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Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation

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ABSTRACT

World's energy demand has been constantly increasing for decades. Yet, fossil fuels stock, which are used the most extensively nowadays, may be depleted soon. Moreover, combustion of conventional fuels leads to excessive carbon dioxide emission. This process causes multitude of unfavorable consequences for Earth's climate and biosphere. That is why the alternative fuel sources are searched and studied for years. One of the possibilities of obtaining renewable energy is biogas production from biomass through anaerobic digestion process. Anaerobic digestion is widely applied to treat various wastes and higher plants biomass. Laboratory-scale studies proved that microalgal biomass is also a feasible source of substrate for methane fermentation process. Microalgae are predominantly single cell photoautotrophic organisms that have the ability to proliferate rapidly and absorb significant amounts of carbon dioxide at the same time. This characteristics indicate that these microorganisms can be an efficient source of biomass for biogas acquiring. Current study constitutes a comprehensive review which compares and summarizes studies concerning anaerobic digestion of microalgal biomass, specific factors and potential inhibitors that influence the process as well as it presents results of empirical studies. This review is based on the latest publications as well as on older esteemed literature.

Contents

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1. Introduction

According to International Energy Outlook 2011 [1], total world energy use rises from 505 quadrillion British thermal units (Btu) in 2008 to 619 quadrillion Btu in 2020 and 770 quadrillion Btu in 2035 (by 53%). To reduce dependence on fossil fuels and to reduce climate change, there is a need to make a switch to alternative, renewable energy system [2]. Among renewable energy sources like sun, wind, hydroelectric and nuclear energy, biomass is a very promising source of bioenergy. Energy from biomass is regarded as one of the most important future renewable energy sources, because it can provide a continuous power generation and it plays an important role in the current CO_2-mitigation policy [3]. Several biological processes to convert biomass to energy, and thus provide a source of biofuel, have been studied in recent years. One of the most important processes of biomass conversion is the anaerobic digestion (methane fermentation) of organic matter to produce biogas, consisting mainly of methane and carbon dioxide [4]. This is the process of biochemical conversion of organic matter into biogas as a product of the metabolic action of methanogenic bacteria [3–5]. When biogas is used to generate energy, it is possible to generate from 20 to near to 300 kW h of net energy per tonne of waste [6]. Many calculations have shown that the capture of CO_2 and energy recovery from biogas may considerably contribute to greenhouse gases (GHG) emission reductions [7] and at the same time it does not contribute to ozone depletion or acid rain formation [8]. Another reason for utilizing biomass to generate biogas is that the solid waste product from anaerobic degradation (digestate) contains remineralised nitrogen and phosphorus and thus can be used as organic fertilizer in terms of its availability to plants [4,9]. In recent years, research into the potential biomass feedstock for methane fermentation from different sources has been conducted [10]. Anaerobic digestion technologies show great adaptability to a broad spectrum of different input materials. Organic fraction of municipal solid waste, agricultural and animal wastes, sewage sludge and other sources could be used [7]. Currently, scientists all over the world have been examining the possibilities of using microalgae as a source of biogas for energy applications [10]. In Table 1 a quick overview and comparison of methane yields obtained from different types of biomass and microalgae are included. The aim of the current study is to present anaerobic digestion process general characteristics and inhibitors and confront it with the possibility of the anoxic digestion of microalgal biomass. The study summarizes and compares the results of empirical studies of biogas productivity from the sole biomass as well as from microalgal post-extraction remains and biomass co-digestion with other substrates.

Table 1
Comparison of methane yields obtained through anaerobic digestion of various substrates and microalgae (adapted from [8] and modified).

Substrate for biogas production	Methane yield ($m^3\ kg^{-1}$ VS)
Municipal solid waste	0.20–0.53
Fruit and vegetable wastes	0.42
Jatropha oil seed cake	0.42
Swine manure	0.34
Maize silage and straw	0.31
Microalgae[a]	0.26
Lignin-rich organic waste	0.20

[a] Methane yield from microalgae is calculated as a mean from data gathered in Table 5; data regard only to digestion at circa 35 °C and during HRT of 30 days.

2. Anaerobic digestion of microalgae

Anaerobic digestion is the process of decomposition of organic matter by a microbial consortium in an oxygen-free environment [9]. Anaerobic digestion involves a series of metabolic reactions such as hydrolysis, acidification, acetogenesis and methanogenesis which are conducted by various groups of microorganisms [8]. The first group of microorganisms enzymatically hydrolyze complex organic compounds to monomers (e.g. glucose, amino acids), which are subsequently converted to higher volatile fatty acids (VFA), H_2 and acetic acid. Then, the acetogens convert higher volatile fatty acids e.g., propionic and butyric acid, produced, to H_2, CO_2, and acetic acid. Eventually, methanogenic bacteria convert H_2, CO_2, and acetate to CH_4 and CO_2 [11]. Typical composition of biogas produced is shown in Table 2.

Anaerobic degradation of phytoplanktonic cells is a process which occurs in natural water reservoirs. When algal cells sink towards the anoxic and aphotic zones of the reservoir, they eventually die and become a part of bottom deposits. The algal debris then undergoes fermentation. It leads to ammonium and phosphate but simultaneously toxic substances release, such as H_2S. This process can deplete stream oxygen reserves at night. Such depletion is harmful to fish and other wild life in the stream, and therefore must be avoided [12,13]. The total estimated biogenic CH_4 emission to the atmosphere is approximately 525–715 $\times 10^6$ t per year and this rate is rising by about 1% per year [14,15].

This potential can be exploited for the production of chemical energy of methane through the fully-controlled combined algal-bacterial anaerobic digestion process. Microalgal biomass production is the first step of the process [16]. The algal biomass production potential and varied algae cultivation methods are widely reported in literature [10,17–19] and thus this subject will not be covered in the current study. Next, the biomass is used as nutrient (as a batch) for feeding the anaerobic bacteria for the production of methane [16].

Table 2
Composition of biogas generated by anaerobic digestion [6].

Compound	Biogas concentration (%)
CO_2	25–50
Methane	50–75
Water	6–6.5
O_2	0.9–1.1
N_2	3.9–4.1
H_2	
H_2S	$<$ 0.1–0.8
Ammonia	$<$ 0.1–1
Mercaptane	In spores
Low molecular fatty acids	
Higher molecular substances	Traces

Table 3
Effect of low nitrogen growth conditions on the composition of three *Chlorella* species and estimation of the theoretical methane potential (in brackets: computed theoretical methane potential of the residual biomass after lipid extraction) [13,24], modified.

	Protein (%)	Carbohydrate (%)	Lipid (%)	Calorific value ($kJ\,g^{-1}$)	CH_4 (L CH_4 g VS^{-1})
Chlorella vulgaris (control)	29	51	18	18	0.64 (0.56)
Chlorella vulgaris (low N)	7	55	40	23	0.69 (0.48)
Chlorella emersonii (control)	32	41	29	21	0.74 (0.62)
Chlorella emersonii (low N)	28	11	63	29	0.92 (0.76)
Chlorella protothecoides (control)	38	52	11	19	0.65 (0.60)
Chlorella protothecoides (low N)	36	41	23	24	0.71 (0.62)

Table 4
Specific methane yield for three types of organic compounds [13,27,29], modified.

Substrate	Composition	g COD · g-VS^{-1}	L CH_4 · g-VS^{-1}	CH_4 (%)
Proteins	$C_5H_7NO_2$	1.42	0.446–0.496	50
Lipids	$C_{57}H_{104}O_6$	2.90	1.014	70
Carbohydrates	$(C_6H_{10}O_5)n$	1.19	0.415	50

Cultivation of algal biomass requires large amounts of fertilizers and also the waste management of residual biomass (e.g. after lipid or high-value products extraction) has to be considered. Anaerobic digestion can solve these issues and balance the economic and energetic incomes and expenses. Nitrogen and phosphorus remineralisation occurs during the process [13], so the residuals remaining after anaerobic digestion could either be recycled as nutrients for algal cultivation or could be sold as soil fertilizers and conditioners [20]. Moreover, digestion of microalgal biomass releases low amount of hydrogen sulphide in comparison to other types of substrates because of only minimal amount of sulphurated amino acids in their proteins [13].

There were some experiments on anaerobic decomposition of microalgal biomass conducted. On the basis of these studies, the overall view of process characteristics and potential inhibitions jeopardizing the process are described below.

2.1. Substrate composition

Chemical, physical as well as biological properties of produced algal biomass are dependent on cultivation conditions under which it is grown and simultaneously they determine the anaerobic process run. These properties can be indirectly influenced by inducing either optimal and/or stress cultivation conditions e.g. nitrogen, silicon and phosphate depletion stress, modified light intensity, high salinity and other [21,22].

Cell composition is strongly species dependent but it is also deeply affected by environmental conditions [13]. Species classified under *Chlorophyceae*, *Volvocales* (*Chlorella* sp., *Botryococcus braunii*, *Dunaliella salina*) exhibit typical biochemical composition of 30–50% proteins, 20–40% carbohydrates and 8–15% lipids. Under stress conditions these species are able to accumulate up to 80% of fatty acids, 80% of hydrocarbons, and 40% of glycerol [23]. Changes in the biochemical composition of cells exposed to optimal conditions and nitrogen deficiency stress are presented in Table 3. In regard to elemental composition, cells consist of 50% of carbon, 1–10% of nitrogen and less than 1% of phosphorus [25]. For example, in the study of Golueke et al. [12], microalgae harvested from natural lagoons, consisting principally of *Scenedesmus* spp. and *Chlorella* spp., had total nitrogen content from 6 to 8% of which 3% was in the form of ammonia.

2.1.1. Theoretical biogas potential and modeling

Methane production is directly related to organic degradation [26]. When organic material is degraded anaerobically, the end result is carbon in its most oxidized form (CO_2) and its most reduced form (CH_4). Hence, anaerobic digestion is the most complete of all fermentation processes because methane cannot be further reduced and carbon dioxide cannot be further oxidized. The ratio between CH_4 and CO_2 depends on the oxidation state of the carbon present in the organic material, thus the more reduced the organic carbon content is, the more CH_4 will be produced [27]. For a compound $C_aH_bO_cN_d$ the anaerobic digestion process can be written as Eq. (1) [13,27,28]:

$$C_aH_bO_cN_d + 1/4(4a-b-2c+3d)H_2O \rightarrow 1/8(4a-b+2c+3d)CO_2 + 1/8(4a+b-2c-3d)CH_4 + dNH_3 \quad (1)$$

However, it has to be emphasized that this equation does not take cell metabolism into account. If the composition of the organic material is known and provided that all the material is converted to biogas, the theoretical methane yield potential can be calculated from the Buswell's Eq. (2) which assesses the specific methane yield in standard temperature and pressure (STP) and is usually expressed as STP l-CH_4 g-VS^{-1} (volatile solids) [27]:

$$B_{o,th} = \frac{\left(\frac{a}{2}+\frac{b}{8}-\frac{c}{4}\right)22.4}{12a+b+16c} \left(STP\frac{lCH_4}{gVS}\right) \quad (2)$$

where 22.4 is the volume of 1 mol of gas (methane) at STP conditions [27]. Table 4 presents methane yield in relation to various types of organic compounds.

Theoretical methane yield might be also calculated from the chemical oxygen demand (COD) of the substrate. The COD test measures the organic matter concentration by measuring the oxidant (e.g. dichromate) consumption for the oxidation of the organic material in aerobic conditions [28]. Organic matter consisting of only C, H, and O is theoretically fully oxidized to CO_2 and H_2O. The theoretical COD value of a specific compound can be calculated from stoichiometric considerations. If this theoretical value corresponds to the experimental value, it could be concluded that the oxidation of the organic material is complete [27]. Biogas production in relation to COD is about 0.5 l g^{-1} COD removed, corresponding to a methane production of approximately 0.35 l CH_4 per g of COD removed (3) [27,30].

$$COD_{substrate} \times 0.35\left(STP\frac{lCH_4}{gCOD_{substrate}}\right) = theoretical methane yield (lCH_4 g-VS^{-1}) \quad (3)$$

The COD of methane is 4 mg mg CH_4^{-1} and free energy release from the oxidation of methane per 1 g COD is 12.52 kJ [28]. COD is also a good indicator of the progress of the degradation during process, as any undigested material will require oxygen (in an aerobic environment) to complete degradation [9].

In order to predict and understand anaerobic digestion better, mathematical modeling of the process has been developed. The first simple models date back to the mid-sixties [31]. Nevertheless, as the knowledge of methane fermentation has increased, more complex and realistic models have been evolved. They have included more and more specific reactions of anaerobic digestion process and a range of factors which can influence digestion efficiency [32]. Further model development has addressed adaptation the digestion to specific types of substrates e.g. manure,

sludge, wastewater [31]. Currently, the most approved and comprehensive, generalized model of anaerobic digestion is ADM1 (Anaerobic Digestion Model No 1) which was developed by The IWA Anaerobic Digestion Modelling Task Group, and the report concerning its work was published by Batstone et al. [33,34]. The structured model includes multiple steps describing biochemical (19 reactions) as well as physicochemical processes. It is assumed that the latter are independent of microorganisms. The model is based on degradable COD as a substrate [33,34]. Detailed description of the variables, equations and kinetics as well as structured model of the reaction paths are included in Batstone et al. [33,34].

There were few studies concerning fitting this model to anaerobic digestion of microalgae [35]. The authors have adjusted the model to suit microalgal process best. As the hydrolysis is a limiting step, they have used Contois model for it, which assumes that the kinetics do not depend on the substrate concentration, but on the amount of substrate per biomass unit. The authors conclude that this submitted model fits very well the data provided by a 140 day experiment of *Chlorella vulgaris* digestion [36].

2.2. Process temperature

There are different fermentation techniques applied, which are distinguished mainly on the basis of the operating temperature [6]. Three temperature regimes can be used in anaerobic digesters: psychrophilic, mesophilic, and thermophilic with varied optimum temperature ranges for the domination of different strains of methane-forming bacteria [37]. Psychrophilic fermentors operate at about 25 °C, mesophilic ones at around 35 °C and thermophilic—at around 55 °C. Generally, an increased temperature has a positive effect on the metabolic rate of the microorganisms and the process runs faster, but the thermophilic process is harder to control and needs more energy to keep the fermenter at the fixed temperature [7,38]. At 55 °C more rapid degradation of fatty acids was found than at 38 °C as well as retention time was shorter as 95% of the methane yield was obtained after 11 days under thermophilic conditions compared to 27 days under mesophilic conditions [9]. Chae et al. [39] reported that biogas production rate was reduced due to even small changes in temperature and Appels et al. [40] claims that the process can fail at temperature fluctuations of even 1 °C per day. Ehimen et al. [41] reported an increase of 54–61% in CH_4 yield from algal remnants when temperature of the process was increased from 25 to 35 °C.

2.3. Water content

Anaerobic plants can also be classified on the basis of water content of the batch. Dry systems operate with 30–40% dry matter in the feedstock, and wet systems operate with 10–25% dry matter [7]. It has been reported that the highest methane production rates occur at 60–80% of humidity [8]. However, biomass sources containing much water (even containing less than 40% dry matter) can be processed without any pretreatment [3,9].

Fresh algal slurry after harvesting contains only 2–10% of dry mass [12,42,43]. Thus, the costs of downstream processing in e.g. biodiesel production is very high because of the high energy consumption for drying, extraction and conversion processes. The use of anaerobic digestion technology eliminates these obstacles because the downstream processes of the algal slurry are minimal [20].

2.4. 4 pH

The next factor which influences the fermentation process is pH. As a rule, the most favorable range of pH to achieve maximal biogas yield in anaerobic digestion is 6.8–7.2 [37]. Methanogenic bacteria are extremely sensitive to pH fluctuations and prefer pH around 7.0 as the growth rate of methanogens is greatly reduced below pH 6.6 [9,37]. Acid-forming bacteria are less sensitive and tolerate pH in range of 4.0–8.5, but the optimal pH for hydrolysis and acidogenesis is between 5.5 and 6.5 [9,37,40]. Therefore, some designers prefer the isolation of the hydrolysis/acidification and acetogenesis/methanogenesis processes in two separate stages [9]. At the beginning of the fermentation, acidogens and acetogens produce acids and CO_2 and, as a consequence, pH decreases. Afterwards, the methane-producing bacteria consume the acids, the pH of the digester increases and then stabilizes [37]. This factor is also important because it influences the proportion of ionized and non-ionized forms of inhibitors of methanogenesis. Excessive fatty acids, hydrogen sulphide, and ammonia are toxic only in their non-ionised forms (FA and H_2S–pH below 7, NH_3–pH above 7) [9].

2.5. C/N ratio

The factor that is inseparable with pH is carbon to nitrogen ratio (C/N ratio). The optimal C/N ratio for anaerobic degradation of organic waste is 20–35. When the C/N ratio is lower, it means that the material is protein (and nitrogen) rich. Anaerobic digestion of such material results in increased content of free ammonia released [8]. For some species of algae the high proportion in proteins is characterized by a low C/N ratio if compared with terrestrial plants. Carbon to nitrogen ratio of freshwater microalgae on average is 10.2 while terrestrial plants has this ratio of about 36 [13]. In empirical studies, authors report that e.g. carbon to nitrogen ratio of *Spirulina maxima* biomass was very low – 4.2 [44], algae from natural reservoir (mainly *Chlorella* sp. and *Scenedesmus* sp.) – 6.7 [45], *Chlorella* post-extraction residues – 5.4 [41] and similar (Table 5). It was found that fermentation of such substrates releases great amount of ammonia (7000 mg l^{-1}) as well as large amount of volatile fatty acids was observed at the same time, as a consequence of the toxic effect of ammonia on the anaerobic bacteria [13]. This factor greatly influences pH fluctuations due to ammonia release which increases the pH and inhibition that results with VFA content increase and pH lowering at the same time.

The two-stage reactor with biomass retention has been reported to be considered the only type capable of reliable activity with C/N ratios less than 20 [9]. To overcome this obstacle and increase C/N ratio, co-digestion with low-proteinaceous substrate in appropriate ratio is applied.

2.6. Organic loading rate

The organic loading rate (OLR) is the amount of volatile solids to be fed into the digester each day in continuous process. As the organic loading rate increases, the biogas yield increases to some extend but above the optimal OLR the volatile solids degradation and biogas yield decreases due to overloading [46,47]. On first days of the fermentation, the addition of a large volume of new material each day may cause a significant change in digester's environment and temporarily inhibit bacterial activity [12]. It happens due to the fact that hydrolysis/acidification bacteria can produce to much volatile fatty acids from excessive substrate in a short time. As a consequence, it lowers the pH of the digester and methane-forming bacteria are not able to convert so much acids to methane. For example, in study of Ehimen et al. [41] the most effective organic loading rate was 5 g VS l^{-1}. At higher loading rates an increase in the valeric and butyric acids content occurred, which resulted in inhibition. De Schamphelaire and Verstraete [48] received higher biogas yields when loading rate was the lowest (0.6 g VS l^{-1} AD sludge).

Table 5
Summary of the results of empirical studies of anaerobic digestion of sole and whole microalgal biomass.

Species	C/N	Temp. [°C]	Methane yield [m^3 kg^{-1} VS introduced]	HRT	References
Scenedesmus spp., *Chlorella* spp., mixed, harvested from natural lagoon	–	35	0.31	30	Golueke et al. [12]
Scenedesmus spp., *Chlorella* spp., mixed, harvested from natural lagoon	–	50	0.32	30	Golueke et al. [12]
Spirulina maxima	4.2	35	0.31	20	Samson and LeDuy [44]
Nondefined mixed culture dominated by *Chlorella*	–	34	0.35 0.44 0.60 (biogas containing 40–65% methane)	14 25 45	De Schamphelaire and Verstraete [48]
Nondefined mixed culture dominated by *Chlorella*	–	41	0.28–0.35 0.39–0.47 (biogas containing 40–65% methane)	14 25	De Schamphelaire and Verstraete [48]
Scenedesmus spp. and *Chlorella* spp.	6.7	35	0.10–0.14 (573 ml l^{-1} day^{-1})	10	Yen and Brune [45]
Non-axenic culture of *Scenedesmus obliquus*	–	33	0.21	30	Zamalloa et al. [64]
Non-axenic culture of *Phaeodactylum tricornutum*	–	33	0.35	30	Zamalloa et al. [64]
Non-axenic culture of *Scenedesmus obliquus*	–	33 54	0.13 0.17	2.2	Zamalloa et al. [64]
Non-axenic culture of *Phaeodactylum tricornutum*	–	33 54	0.27 0.29	2.2	Zamalloa et al. [64]
Chlorella vulgaris	6	35	0.24	28	Ras et al. [36]
Chlorella vulgaris	6	35	0.147	16	Ras et al. [36]
Arthrospira platensis	–	38	0.293	32	Mussgnug et al. [62]
Chlamydomonas reinhardtii	–	38	0.387	32	Mussgnug et al. [62]
Chlorella kessleri	–	38	0.218	32	Mussgnug et al. [62]
Dunaliella salina	–	38	0.323	32	Mussgnug et al. [62]
Euglena gracilis	–	38	0.325	32	Mussgnug et al. [62]
Scenedesmus obliquus	–	38	0.178	32	Mussgnug et al. [62]
Microcystis sp. from Taihu lake	6	35	0.201	30	Zhong et al. [76]
Microcystis sp. from Taihu lake	–	35	0.14	30	Zeng et al. [53]
Species unknown	–	30	929–1294 ml of biogas	28	Salerno et al. [54]
Chlorella vulgaris	–	37	0.286	49	Lakaniemi et al. [61]
Dunaliella tertiolecta	–	37	0.024	49	Lakaniemi et al. [61]

2.7. Retention time

Retention time is the time required to degrade the organic matter completely and it is connected with microbial growth rate. The retention time depends on process temperature and batch composition. The average retention time for waste treated in a mesophilic plant is 15–30 days and a bit shorter for thermophilic plant [49]. There are two significant types of retention time – the solid retention time (SRT) – the average time the bacteria (solids) are in the anaerobic digester, and hydraulic retention time (HRT). HRT is defined by following Eq. (4) [50]:

$$HRT = \frac{V}{Q} \tag{4}$$

V is the volume of the biological reactor and Q the influent flow rate in time

Digestion time is one of the main factors influencing the CH_4 yield [41]. Effective hydraulic retention time depends on the type of substrate and on loading rate, and reaches up to a couple of weeks. Shorter HRT usually results in accumulation of VFA, whereas at HRT longer than optimal, the digester components are not effectively utilized [51]. If the HRT is short and bacterial loss exceeds the growth rate of bacteria, 'wash-out' occurs and the process can fail [37,52].

As far as algal biomass is considered, HRT below 10 days results is low methane productivity (Table 5). According to Ras et al. [36], methane productions from microalgae as a function of time can be fit in inverse exponential curve, characterized by an increase between 10 and 30 days HRT and reaching a stable level after 30th day. Summing up, when the process is operated at low loading rate and long hydraulic retention time, methane yield is constant and maximal [13].

2.8. Inoculum to substrate ratio

In case of batch process, Zeng et al. [53] investigated inoculum to substrate ratio (ISR) (based on volatile solids) as a key factor of methane productivity from algal biomass. It was reported, that cumulative methane yield was the highest when ISR was 2.0 while the percentage of methane in biogas volume was increased up to 45% as the ISR decreased to 0.5.

3. Inhibition of the process

Some bottlenecks are identified to digest microalgae anaerobically. Inhibitory factors cause an adverse shift in the microbial population or limitation of bacterial growth. Inhibition is often indicated by a reduction of the steady-state rate of methane production and accumulation of organic acids [13,38]. In one of the oldest reports [12] authors mention that amount of destructed volatile matter and gas produced from algae were always lower that obtained from raw sewage sludge fermentation. Methane conversion efficiencies of microalgal biomass and raw sewage sludge reached 36.4–44.3% and 57–60% in case of mesophilic fermentation, respectively. In the same study authors also report that digested algae had some undesirable physical characteristics—sludge was highly colloidal, gelatinous and poorly dewatered as well as had the odor of fresh cow manure. It appears that microalgal biomass either require more time to degrade or it cannot further degrade [36]. Some inhibition factors could be reduced by various operations, e.g. pretreatment of the substrate or co-digestion with other material. Acclimation of the digester microbial community to microalgal biomass digestion may also improve the methane yield [54].

3.1. Ammonia

Ammonia is an important source of nitrogen for bacteria and low concentrations of ammonia (below 200 mg l^{-1}) are beneficial to the process [38]. However, it has been found that the specific activity of methanogenic bacteria decreases with increasing concentrations of ammonia [8]. There are several mechanisms responsible for ammonia inhibition: change in the intracellular pH, increase of maintenance energy requirement as well as inhibition of a specific enzyme reaction [38]. Moreover, high ammonia concentration in the digester decreases the deamination activity of proteolytic bacteria [55]. Free ammonia seems to be the main cause of inhibition because it is freely, passively membrane-permeable and causes proton imbalance and/or potassium deficiency [38]. An increase in pH results in the shift of ionized form (NH_4^+) to free ammonia and increased toxicity. Process instability due to ammonia often results in volatile fatty acids accumulation, which leads to a decrease in pH and thereby declining concentration of free ammonia. This interaction may lead to an 'inhibited steady state', a condition where the process is running stably but with a reduced biomethane yield [38]. Higher process temperature also results in a higher excretion of free ammonia. Chen et al. [38] reports that decrease in operating temperature from 60 to 37 °C in anaerobic digesters with a high ammonia concentration provided an increase in biogas yield.

However, methanogens can adapt to ammonia concentrations above 1700 mg l^{-1} without the occurrence of a lag-phase if ammonium-nitrogen concentration increases slowly [38,56]. In study of Yang et al. [57] repeated batch fermentation was applied as a method of adaptation methanogens to high-proteinaceous algal biomass. Methane production rate was increased from 17 ml CH_4 day^{-1} to 28 ml CH_4 day^{-1}, but on the other hand, ammonia was being accumulated in the fermenter and slightly lowered methane yield in every subsequent cycle.

Highly proteinaceous composition of algae also contribute to formation of a digested sludge with very low C/N ratio—5 [12].

3.2. Sulfur

Sulfur is an element required for methanogenic bacteria, and moreover cells of the methanogens contain more sulfur than other groups of anaerobic organisms [38]. Although, sulfur – in form of sulfate or sulfide – may become an inhibitor in the anaerobic digestion process. As sulfate is reduced by sulfate reducing bacteria, competition for the substrates between sulfate reducing bacteria and methanogenic bacteria can constitute inhibition. Furthermore, the inhibition results from the toxicity of sulfide and produced hydrogen sulfide to other groups of bacteria. H_2S is toxic because it diffuses into the cytoplasm by cell membranes and may form disulfide cross-links between polypeptide chains and denaturate the proteins. It was also observed that toxicity of sulfide increases with pH [38]. Sulfur content of microalgal cells is rather low and ranges between 1.5 and 16 μg mg^{-1} dry weight [25] which corresponds to 0.15–1.96% by dry weight [58–60].

3.3. Macroelements, microelements, heavy metals

Supplementation of certain metals increases biogas production, due to the fact that some metals are used as a part of the enzymes structure of the bacteria [9]. On the other hand, some macroelements, microelements and heavy metals may have toxic effect on anaerobic microflora in digester. High concentrations of essential alkali metals like magnesium, calcium, sodium, and potassium can be toxic to anaerobic bacteria [37]. Excessive amounts of calcium result in precipitation of carbonate and phosphate and cause scaling of reactors as well as of bacterial cells. Scaled biomass is less active because of mass transfer limitations. Excessive potassium leads to passive influx of K-ions and neutralize the membrane potential [38]. In case of marine algae especially, high concentration of sodium in their biomass might be an inhibitor of the anaerobic digestion due to osmotic stress and dehydratation [13,38]. However, in study of Lakaniemi et al. [61] it turned out that the inhibiting element of fermentation of marine species *Dunaliella tertiolecta* could be rather chloride than sodium. Authors mention that on freeze-dried biomass of this species, salts on the surface of the biomass were visible. However, anaerobic microflora is feasible to adapt to salt environment and inhibiting effect may not occur [13]. Acclimation of methanogens to sodium can shorten the lag phase before biogas production begins [38].

Ogejo et al. [37] reports that digesters treating municipal wastewater have failed sometimes because of copper, zinc, chromium, and nickel were present in the substrate. Toxicity of heavy metals results from that heavy metals are not biodegradable, thus may accumulate to toxic concentrations and negatively affect enzyme function [38]. According to Golueke et al. [12], aluminum in algae harvested by alum-flocculation had no apparent inhibitory effect on digester activity, but on the other hand Chen et al. [38] mentions that aluminum was reported to inhibit the growth of methanogens and to reduce biogas production and methane content in the biogas.

3.4. Fatty acids

Short chain—volatile fatty acids are not toxic themselves. They are produced and used as nutrients normally in an active digester. However, their inhibiting effects could be indirect as they might lower the pH to undesirable level [12]. The methanogens will not be able to metabolise the acetate produced by the acetogenic organisms until the number of methanogenic organisms has increased sufficiently. This is especially true of feedstocks which are rapidly hydrolyzed [9]. Increase of VFA content may be caused by increased activity of the acidogenic bacteria coupled with inhibition of methanogens. Various acids concentrations are good indicators of the digester condition. It was demonstrated that propionic to acetic acid ratio >1.4 and acetic acid content of >800 mg l^{-1} is a signal of digester failure. The accumulation of butyric and valeric acid also results in inhibition of the process and cause digester failure at >6500 mg l^{-1}. The VFA to alkalinity ratio adequately characterizes the digestion process: the lower ratio, the higher methane yield is [41].

Long chain fatty acids can also constitute inhibition to anaerobic digestion process. Long chain fatty acids retard gram-positive bacteria' activity, which includes methanogens. Toxicity of long chain fatty acids results from adsorption onto the cell wall or membrane, causing disorientation of essential groups on the cell membrane and thus transport and protection function problems [38,55]. This factor must be considered as far as microalgal biomass can reach high content of lipids if cells are cultivated in stress conditions.

3.5. Cell wall

Cell wall is considered to be the main characteristics of digestion capability of algal biomass [62–64]. Microscopic analysis of Golueke et al. [12] showed that a large amount of algal cells in the sludge, from both thermophilic and mesophilic experiments, were intact (but not viable) which indicates that probably cell wall of algae inhibited the microbial penetration. It was also reported that photosynthetic activity of the intact algae in the digester can cause the presence of oxygen in the biogas [13], that is why the digesters should be kept in the darkness when microalgal biomass is digested [36]. However, Mussgnug et al. [62] notes that even

after six months after transfer, there were intact cells of *Scenedesmus obliquus* present in the digester, which indicates that this species not only has resistant cell wall but is also probably able to utilize variety of sugars and organic acids for heterotrophic growth.

In general, algal cell walls are made up of two components: the fibrillar component, which forms the skeleton of the wall (cellulose, mannan, xylans), and the amorphous component, which forms a matrix within which the fibrillar component is submerged [65]. Ultrastructure of the cell walls of genus *Chlorella* and *Scenedesmus* (*Chlorococcales*) are well studied. Cell walls of these genera comprise of two layers. The inner layer of the cell wall consists of cellulose microfibrils and amorphous matrix and outer layer may appear in two forms—homogenous or as trilaminar sheath (TLS) [66]. The trilaminar component includes a substance considered previously to be the polymerized carotenoid, sporopollenin [67]. Sporopollenin is an UV autofluorescent lignin-like biopolymer surrounding the zygotes of several charophytic algae species, such as *Coleochaete*, and constituting the outer wall of noneuphyllophyte spores and euphyllophyte pollen [68]. Sporopollenin is polymerized from hydroxylated fatty acids and phenolics. Nowadays sporopollenin-like substances in algal cell walls are called algaenan or acetolysis-resistant biopolymer (ARB). Algaenan is resistant to extreme extraction procedures including acetolysis [67] as well as to organic solvents, bases and non-oxidizing acids [66]. In studies conducted by Gunninson and Alexander [69], cell walls of the algae were fractionated, and the fractions least susceptible to microbial degradation were the sporopollenin-like substance.

On the other hand, Grossi et al. [70] in the study conclude that presence of algaenan-containing cell wall do not protect other components of the cell from microbial degradation under either oxic or anaerobic conditions.

One of the main limits on the anaerobic digestion process, as far as higher plant are considered, is its inability to degrade lignin (a major component of wood). This is in contrast with the process of aerobic biodegradation [6]. The chemical composition and structure of lignocellulosic materials lowers the rate of biodegradation [8]. Lignin has generally been considered to be a hallmark of tracheophytes, but there are sporadic reports in the literature describing the detection of lignin or 'lignin-like' compounds from nonvascular plants, including brown algae, charophytic algae and mosses [68]. Also lignin derivatives with aldehyde groups or apolar substituents may affect methanogens [38]. The most popular species for cultivation like *Chlorella* are believed to produce no lignin and only a little cellulose or other carbohydrate wall material [65,68]. However, Sui et al. [71] analyzed lignin content in *Chlorella* cell walls and the results appeared to be unexpected. Two analysis methods were used and gave values of 2.45–6.35 wt% (of extract-free cells). Both analyses seem to indicate the presence of phenylpropane units, but that conclusion has not been confirmed.

3.5.1. Pretreatment

Various physical, chemical and enzymatic pretreatments are used in order to increase substrate solubility and accelerate the biodegradation rate of solid organic waste [8]. These methods of pretreatment can also significantly and efficiently increase the conversion yield of the algal organic matter into methane by increasing the accessibility to algal protoplast [13]. There were some studies concerning pretreatment of microalgal biomass for anaerobic digestion carried out [63,72–75]. A few methods of pretreatment were examined, e.g. thermal, ultrasound, pressing, chemical, enzymatic. Most of the results indicate that pretreatment is a crucial step of the whole process and the yields of biogas and methane are higher after the pretreatment is applied. Subjecting biomass to physicochemical treatment weakens the rigid cell wall structure and thus allows methanogens to consume the organic compounds inside the cell. Moreover, smaller particles, with higher surface area to volume ratios, will have higher reaction efficiency during anaerobic digestion for biogas [20]. Yang et al. [57] state that two-stage process of hydrogen and methane production can be a promising idea of energy acquiring as methane yields are higher compared to regular one-step anaerobic digestion. Hydrogen production before anaerobic digestion process can play as a kind of pretreatment step for methanogenic bacteria, because it enhances hydrolysis and improves biodegrability of algal remnants. One of the exceptions is drying process which decreases fermentative potential of microalgal biomass [62]. It must be noticed that excessive energy input for pretreatment might negatively impact the economic feasibility of the whole process of biofuel production [55].

Table 5 shows the results of empirical studies conducted concerning anaerobic digestion of the whole algal biomass.

4. Co-digestion

Co-digestion is a substrate treatment method in which different substrates are mixed and treated together. It is also termed as 'co-fermentation'. Co-digestion is an energy-efficient process that can improve fermentation performance by adding a secondary substrate that supplies nutrients that initial substrate is lacking, improving physicochemical parameters of the batch [55]. Co-digestion is

Table 6
Summary of the results of empirical studies of co-digestion of microalgal biomass with other substrates.

Species	Co-substrate	C/N	Temp. [°C]	Methane yield [$m^3\ kg^{-1}$ VS introduced]	HRT	References
Spirulina maxima	Primary domestic sewage sludge 49.4%	6.2	35	0.36	20	Samson and LeDuy [44]
Spirulina maxima	Peat hydrolyzate 35%	6.3	35	0.28	20	Samson and LeDuy [44]
Spirulina maxima	Spent sulfite liquor 9.3%	4.7	35	0.25	20	Samson and LeDuy [44]
Chlorella post transesterified residues	Glycerol	8.53	40	0.308	15	Ehimen et al. [41]
Chlorella post transesterified residues	Glycerol	12.44	35	0.295	15	Ehimen et al. [41]
Scenedesmus spp. and *Chlorella* spp.	Waste paper 50%	18	35	1170 ml l^{-1} day^{-1}	10	Yen and Brune [45]
Post-lipid-extracted residues of *Nannochloropsis salina*	Lipid-rich fat, oil and grease waste 50%	-	37	0.54	27	Park and Li [55]
Microcystis sp. from Taihu lake	Corn straw	20	35	0.325	30	Zhong et al. [76]
Species unknown	18 ml algae +0.5 ml soybean oil	–	30	1794 ml of biogas	28	Salerno et al. [54]
Species unknown	18 ml algae +0.082 ml glycerin	–	30	1013 ml of biogas	28	Salerno et al. [54]

preferably used for improving yields of anaerobic digestion due to its numeral benefits. Dilution of toxic compounds, increased load of biodegradable organic matter, alleviating imbalance of nutrients, adjustment of the carbon-to-nitrogen (C/N) ratio, preventing inhibition, synergistic effect of microorganisms, easier handling of mixed substrates, economic advantages due to equipment sharing and better biogas yield are the potential benefits that are achieved in a co-digestion process [8,9,13,55,76]. Well-maintained nutrient balance of the fermenter not only gives higher methane yield but also allows higher loading rates, improving the economic feasibility of the process [55]. Due to the fact that microalgal biomass has a very low C/N ratio (4–6), co-digestion with carbon-rich co-substrate is widely recommended.

In Samson and LeDuy [44] study, spent sulfite liquor (SSL) turned out to be inappropriate as co-substrate. Despite the high C/N ratio, the more SSL was introduced to algal biomass, the less methane was produced or the process failed. Authors did not explain the possible reasons for such case. Yen and Brune [45] observed that C/N ratio 11.8–18.0 is most suitable for anaerobic digestion of algal sludge with waste paper and the achieved biogas production is similar in this range of C/N ratio. The authors also analyzed the cellulase activity in the process, as cellulose hydrolysis is considered the rate-limiting step of the anaerobic digestion. Co-digestion of algal sludge mixed with waste paper (50%/50%) resulted in high cellulase activity and highest methane production. However, digestion of waste paper alone resulted in highest cellulase activity but poor methane production. Therefore, it is assumed that algal biomass contributed some key components that improved activity of methanogens. Simultaneously, increase in cellulase activity might be beneficial for biodegradation of algal cell walls. Gonzáles-Fernández et al. [77] co-digested microalgal biomass with swine manure. However, anaerobic digestion of algae as a sole substrate as well as algae as a predominant co-substrate resulted in low methane productivity (128.9–143.0 mL CH_4 g COD^{-1}) and the highest methane yields were achieved when swine manure was fermented as a sole substrate (245.9 ml CH_4 g COD^{-1}). Microscopic analysis proved that algal species used in the study were mainly *C. vulgaris* and *S. obliquus*. These species are known to have a recalcitrant cell wall and hence it was assumed that the cell wall was responsible for the inhibition of biogas production. In study conducted by Zhong et al. [76], the highest biogas and methane yield was reached by co-digestion of blue algae biomass with corn straw at C/N ratio adjusted to 20. At such C/N ratio, volatile solids reduction (%) and methane content in biogas (%) was also the highest. Increase of methane productivity by 61.69% was noted in comparison to digestion of algal biomass solely. The authors emphasize, that positive synergistic effect occurred due to well-balanced nutrients, increased buffering capacity and probable increased cellulose activity, as a result of co-digestion. Salerno et al. [54] noticed that at first two to three weeks of the experiment concerning co-digestion with soybean oil, kind of inhibition or acclimation occurred and after this period biogas production rate increased abruptly. The same situation appeared when twofold amount of algae was digested. Authors supposed that inhibition occurred due to algal biomass excess.

The results of experiments of co-digestion are presented in Table 6.

5. Anaerobic digestion of algal remnants

Anaerobic digestion can be also effectively used as a means of producing biomethane from remnants which develop after conversion (e.g. extraction) of algal feedstock into fuel [20]. For example, the biodiesel production process results in the co-production of biomass residues and glycerol [41]. As microalgal-derived biodiesel appears to be a promising alternative to petroleum-based liquid fuel, the utilization or disposal of post-extraction residues is a key issue considering balancing both energetic and economic aspects of algal derived biofuel industry commercialization. Post-extraction algal remnants accounts for approximately 65% of the harvested biomass and thus it can generate additional energy through methane fermentation. By integrating anaerobic digestion with algal biodiesel production, whole harvested biomass can be utilized and efficiency can be increased [55]. The economic value of the produced methane is equivalent to about $100 per ton of digested biomass, which is significant in terms of reducing the overall cost of liquid biofuels production [20].

When lipids are extracted from algal biomass before digestion, the potential biogas yield is lower and ammonium release is higher, because of higher protein content by percent. The high ammonium concentration may then strongly limit and even jeopardize the process stability. To manage this rich nitrogen substrate, a co-digestion with a poor nitrogen substrate is thus necessary [13]. An example of such substrate might be another lipid-rich substrates and waste, which offset the C/N ratio imbalance. However, lipids are characterized by low alkalinity and buffering capacity which makes them vulnerable to inhibition. Low alkalinity can be offset by increasing protein content, thus lipid degradation can be also increased due to favorable alkalinity [55].

In the study of Ehimen et al. [41] algal residues were co-digested with glycerol. Thanks to the co-digestion the C/N ratio was increased and simultaneously the CH_4 yield was increased by $>50\%$, although, authors recorded no improvement of biogas productivity with C/N ratio higher than 12.44. In all cases, it was observed that an increase in the digestion time with a corresponding reduction in the loading rates, led to increased CH_4 yields. Unexpected results were achieved by Park and Li [55]. Co-digestion of 50% post-lipid-extracted algal biomass with lipid-rich fat, oil and grease waste gave very high methane yield of 0.54 m^3 kg^{-1} VS. Calculations prove that this result is 23% greater than the theoretical methane potential which indicates that synergetic effect caused by optimal ratio of the substrates occurred.

However, lipid extraction of biomass containing less than 40% of lipids combined with anaerobic digestion of the remnants is not effective in terms of energy nor in term of the costs. When the cell lipid content does not exceed 40%, anaerobic digestion of the whole biomass appears to be the optimal strategy on an energy balance basis, for the energetic recovery of cell biomass [13].

In Table 7 the results of empirical studies concerning anaerobic digestion of algal remnants are presented.

6. Future perspectives

Anaerobic digestion of various organic wastes works well e.g. in Germany which is undisputed leader in biogas production in

Table 7
Summary of the results of empirical studies of anaerobic digestion of algal remnants.

Species	C/N	Temp. [°C]	Methane yield [m^3 kg^{-1} VS introduced]	HRT	References
Post-lipid-extracted residues of *Nannochloropsis salina*		37	0.13	40	Park and Li [55]
Chlorella post transesterified residues	5.4	35	0.245	15	Ehimen et al. [41]
Lipid-extracted microalgal biomass residues from *Scenedesmus*	10.8	37	0.323	50	Yang et al. [57]

Europe [78] or in Sweden which is famous of biogas-supplied city buses. The technology is well known and functions successfully in industry for years. Future research in this field will probably include development of new methods of process monitoring and control (real time measurements), which are lacking at present; improvement of pretreatment and co-digestion methods; and also molecular analyses of microbial community in order to enhance degradation process. There are some studies on immobilization of bacteria as a way to improve reactor efficiency and yields as well as to reduce HRT and possibility of reactor failure. Modernizing the design of plants, in particular mixing systems, is also a vital aspect, as mixing is important for the access of microorganisms to the substrate [8,9,79].

Future perspectives of anaerobic digestion of microalgal biomass are inseparable from cost-effectiveness of the process on industrial scale. Many reports and analyses based on prevailing state-of-the-art technologies and engineering indicate that such system would not be cost-effective. Electric consumption and, associated with it, environmental impact are the main bottlenecks to be overcome. The main trouble spot are immature technologies used for growing and harvesting of algal cells [80]. Particularly, high cost of building and maintaining photobioreactors or ponds and low density of cultures are the weakest points. Improving of pretreatment methods is also a crucial aspect regarding microalgal biomass digestion as many publications report the low bioavailability of resistant compounds of the cells. There is a chance for solving this issue in selection of algal species for cultivation which does not have a cell wall but simultaneously has high growth rate and good biomass composition [36,64]. It is also reported, that viability and sustainability of the process would rise if biogas production from microalgae was coupled with prior biodiesel recovery [13,80,81] or any other valuable product (e.g. pharmaceutics). The solution that may also reduce unfavorable economic and ecological impact is to connect other alternate energy acquiring installations to the whole system, like solar panels or wind turbines [80]. Finally, there is a strong need to develop detailed models and carry out technoeconomic analyses and life-cycle assessments based on various versions and options of the technology in order to discover strong and weak points of the process and suggest advanced, innovative solutions.

7. Conclusions

As it was shown in a few empirical studies, anaerobic digestion of microalgal biomass is possible and gives quite good methane yields. Despite these pleasing results, the process of methane fermentation of microalgae is still not well examined. Many studies are described superficially, with only few aspects of the whole process taken into consideration. Partially, this gap is caused by the fact that cultivation of microalgal biomass is not cost-effective for the time being. Nevertheless, mainly due to its high growth rate, biomass of microalgae is a realistic, alternative biomass feedstock of the future. This indicates that anaerobic digestion of microalgae should be studied more carefully and in detail.

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